



Published in final edited form as:

*Circ Res.* 2018 March 30; 122(7): 925–927. doi:10.1161/CIRCRESAHA.118.312870.

## Immune Modulation as a Treatment for Abdominal Aortic Aneurysms

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### Abstract

In the United States, over 200,000 new patients are diagnosed with abdominal aortic aneurysm (AAA) each year. Consequently, over 40,000 highly morbid aortic reconstructions are performed each year to prevent aneurysm rupture, a catastrophic event associated with near-certain mortality. No pharmaceutical currently exists to slow aneurysm growth, but a 50% reduction in diameter growth per annum could halve the number of aortic reconstructions required. Therefore, successful use of cell therapy to modulate chronic inflammation hallmark to AAA to slow diameter expansion represents a potentially paradigm-altering treatment.

There continues to be no U.S. Food and Drug-approved medication to slow or stop AAA growth. This deficit is certainly not due to a lack of effort over the previous decades. Initial investigations focused on the reduction of mechanical stress through decreasing blood pressure and sac pressurization cycles; however, clinical trials investigating drugs such as angiotensin II converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB), and beta blockers all failed to demonstrate a therapeutic reduction of diameter growth compared to placebo. Next, the inhibition of matrix metalloproteinases (MMP) was trialed with doxycycline, an antibiotic inhibitor of MMP-2 and -9. Doxycycline also treats species of *Chlamydia* which were isolated from the aortic walls of a minority of aneurysmal arteries. While initial uncontrolled results were promising, therapeutic response was not replicated in large randomized studies. Lastly, aortic wall inflammation was targeted using statins, a ubiquitous anti-inflammatory, lipid lowering drug to limited success. The discovery of a nonsurgical treatment for AAA is contingent on the robust understanding of disease pathogenesis, which continues to be nebulous at best.

AAA risk factors such as tobacco abuse, advancing age, uncontrolled hypertension, male gender, and Caucasian ethnicity have all been well described but fail to provide evidence elucidating pathogenesis. Regardless, existing evidence implicates that disease initiation is multifactorial with a strong genetic element. There has been a focus of late toward the role of autoimmune mechanisms in AAA formation. The characterization of aortic wall

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### Disclosures:

The authors have no relevant financial conflicts of interest to the information presented in this manuscript

inflammation consisting of mononuclear infiltrates, immunoglobulins, cytokines, and proteases implicate both a host innate and adaptive response. Of note, concentrations of CD4<sup>+</sup> T cells have been recently discovered in the periadventitial VALT (vascular associated lymphatic tissue) expressing identical T cell receptors, against a currently unknown antigen, and therefore clonally expanded, providing crucial evidence for AAA as an autoimmune disease.<sup>1</sup> Moreover, IL-17, a cytokine elaborated from the antigen-specific Th17 lymphocyte and overexpressed in autoimmune disorders, is highly expressed in the AAA condition. Abrogation of this cytokine, in animal models, have demonstrated efficacy in decreasing aneurysm diameter growth.<sup>2</sup> Although several putative autoantigens have been proposed, the true causative antigen has yet to be identified.<sup>3</sup>

We believe aneurysm initiation can be divided into two stages: sensitization followed by chronic inflammation. In the sensitization phase, elastin from the lung is broken down to small antigenic fragments secondary to injury, often from cigarette smoke exposure and explaining the high risk of AAA formation in those with a significant pack-year history.<sup>4</sup> These previously hidden elastin antigens are key to the progression of COPD severity by recruiting additional inflammatory cells, driving its own upregulation; it also promotes polarization towards the M1 pro-inflammatory macrophage sensitized to elastin-rich tissues such as the aortic wall.<sup>5</sup> Not surprisingly, in serum of patients with AAA compared to risk-factor matched non-AAA controls, we found a significant elevation in IgGs reactive to aortic-derived elastin.<sup>6</sup>

In the inflammation stage, self-sensitized mononuclear cells hone to the infrarenal aorta, a relatively weak section of a large elastic artery prone to excessive mechanical stress secondary to the tapered configuration and shear stress introduced by the aortic bifurcation, exposing previously hidden self-antigens.<sup>7</sup> Additionally, the infrarenal segment is relatively devoid of vascular smooth muscle cells and elastin lamellae in the tunica media, magnifying the impact of any structural damage. Small human aortic aneurysm surgical specimens are histologically characterized by this inflammatory infiltrate which is largely absent from healthy aortic wall. The dominant cell populations are B and T lymphocytes along with smaller populations of macrophages, mast cells, and NK cells. The elaboration of cytokines such as IL-2, IFN $\gamma$ , and TNF $\alpha$  by a predominantly Th1 mononuclear response stimulates pro-inflammatory osteopontin (OPN) secretion from macrophages and vascular smooth muscle cells further propagating the inflammatory response.<sup>8</sup> OPN has been shown to be a strong stimulus for monocyte and lymphocyte chemotaxis, prolongs lymphocyte survival, enhances cell mediated immunity, and polarizes towards the M1 macrophage.<sup>9</sup>

Not all smokers suffer from aneurysmal degeneration of their aortic wall. In our investigations, we found a unique deficiency of the CD4<sup>+</sup>CD49b<sup>+</sup>LAG3<sup>+</sup> Tr1 lymphocyte in the AAA patient which was not present in the circulation of risk-factor matched non-AAA subjects. This antigen-specific regulatory T lymphocyte primarily elaborates IL-10, a potent anti-inflammatory cytokine in response to antigen recognition.<sup>10</sup> Not only was this Tr1 population depleted, but it was also less functional in terms of ability to migrate to a stimulus, secrete IL-10 when activated, and polarize monocytes towards the anti-inflammatory M2 macrophage. Therefore, we hypothesized that this deficit of IL-10 and Tr1, and inability control aortic wall inflammation, may be the driver for initial aortic ectasia

and eventual aneurysm formation. The reason for the depletion is unclear to us presently. However, we hypothesize it may be related to gene suppression via DNA methylation or miRNA interference. In fact, we observed an elevation in miR-15a, miR-27a, and miR-92a, miRNAs known to inhibit IL-10 expression in the plasma of AAA patients.

While chronic inflammation drives initial aortic ectasia and dilation, wall tension consistent with the Law of Laplace becomes the predominant force as the aneurysm continues to expand. When wall tension drives sac expansion, pharmaceutical therapy is no longer an option in AAA treatment and surgical aortic reconstruction is required. Therefore, any pharmaceutical intervention must target the AAA while diameter is still conserved and before wall tension overwhelmingly drives sac expansion. The early identification of patients at risk of AAA development is crucial to the success of nonsurgical treatment. Our preliminary investigations into Tr1 and Th17 expression in the AAA condition have suggested that the ratio between the two may be an accurate predictor of aneurysm presence; however, more studies need to be completed to determine whether this bears out.

Mesenchymal stem cells (MSCs) do not constitutively express MHC-II and therefore represents a potential source of allogeneic donor cells. Although this physiology of this cell population is poorly understood at present, previous studies has shown tissue reparative activity with intravenous infusions of MSCs in the heart, pancreas, and brain undergoing ischemic injury.<sup>11</sup> In our experiments, we found that MSCs, once activated with TNF $\alpha$ , displayed a unique ability to induce the Tr1 lymphocyte *in vitro* through an IL-10 dependent mechanism. Additionally, this induction ability was not cell-cell contact dependent as it occurred even with isolation of MSCs and naïve T-cells on either sides of a transwell membrane. In experiments in the mouse topical elastase AAA model, we observed that human MSCs increased Treg (an antigen nonspecific CD4<sup>+</sup> IL-10 expressing regulatory cell) suppressor function, increased aortic wall Treg counts, and inhibited overall AAA growth over time. Additionally, total IL-10 expression in circulation increased 12-fold over that of controls. Interestingly, mouse IL-10 continued to be elevated over time and continued to peak at day 21 (when the experiment was terminated), even as treatment MSCs were cleared by day 3, suggesting a lasting therapeutic response.

In early 2017, we began enrollment for the phase I ARREST trial (Aortic aneuRysm Repression with mEsenchymal STem cells) targeting patients with small aortic aneurysms (35-45 mm), the only study of its kind.<sup>12</sup> These potential participants are identified through U.S. Preventative Task Force recommended AAA sonographic screenings for patients aged 65, an exam provided for no cost as a part of the “Welcome to Medicare” package. In this study, 36 patients are randomized at a 1:1:1 ratio to placebo,  $1 \times 10^6$  MSCs/kg, or  $3 \times 10^6$  MSCs/kg through a large bore peripheral IV and followed for five years to determine changes in aortic diameter, cytokine expression, and circulating immune cell expression. The aim of treatment is to shift the inflammatory environment of AAA towards that of immune regulation which is observed in the risk-factor matched cohort. We hypothesize that MSC administration will increase Tr1 and Treg suppressor function in a dose dependent manner and upregulate overall IL-10 expression. Currently, seven patients have been enrolled and treated per protocol. Thus far, very preliminary results support an increase in the Tr1:Th17 ratio in response to MSC infusion.

Certainly, challenges with cell therapy have presented themselves as the ARREST trial continues to mature. One of which is finding a steady supply of clinical grade MSCs. These cells must be harvested from the iliac crests of healthy donors, proliferated, and administered at an early passage to ensure consistency and quality. Several times, enrollment was paused secondary to a poor supply of treatment product. Additionally, the treatment product is limited by the possibility of host rejection and hypersensitivity response. Therefore, the MSC exosome represents a particularly interesting treatment product. The exosome is a secreted extracellular vesicle containing mRNA, miRNA, and proteins which incorporates into the target cell membrane to deliver its products to modulate gene expression. MSC exosomes isolated from conditioned media has demonstrated efficacy in multiple animal disease models and could potentially represent a more optimal treatment product for small AAA. It is our belief that non-cellular treatment products, such as exosomes, represents the future in this field as it voids many safety concerns and ensures a homogeneous treatment product.

## Acknowledgments

Funding for ARREST is through a VA Merit Grant (VA CSR&D Merit Award 1I01CX001407-01A1).

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